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(54) Title: ISOFORMS OF THE HUMAN VITAMIN D RECEPTOR

(57) Abstract

The invention provides isolated polynucleotide molecules which encode novel isoforms of the human Vitamin D receptor (hVDR) or variant transcripts for hVDR. These isolated polynucleotide molecules may be utilised in, for example, methods of screening compounds for VDR agonist and/or antagonist activities.

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ISOFORMS OF THE HUMAN VITAMIN D RECEPTOR

Field of the Invention:-

The present invention relates to isolated polynucleotide molecules which encode novel isoforms of the human Vitamin D receptor (hVDR) or variant transcripts for hVDR. The polynucleotide molecules may be utilised in, for example, methods of screening compounds for VDR agonists and/or antagonists.

Background of the Invention:-

The active hormonal form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃), has a central role in calcium and phosphate homeostasis, and the maintenance of bone. Apart from these calcitropic effects, 1,25-(OH)₂D₃ has been shown to play a role in controlling cell growth and differentiation in many target tissues. The effects of 1,25-(OH)₂D₃ are mediated by a specific receptor protein, the vitamin D receptor (VDR), a member of the nuclear receptor superfamily of transcriptional regulators which also includes steroid, thyroid and retinoid receptors as well as a growing number of orphan receptors. Upon binding hormone the VDR regulates gene expression by direct interaction with specific sequence elements in the promotor regions of hormone responsive target genes. This transactivation or repression involves multiple interactions with other protein cofactors, heterodimerisation partners and the transcription machinery.

Although a cDNA encoding the human VDR was cloned in 1988 (1), little has been documented characterising the gene structure and pattern of transcription since that time. The regulation of VDR abundance is one potentially important mechanism for modulating 1,25-(OH)₂D₃ responsiveness in target cells. It is also possible that VDR has a role in non-transcriptional pathways, perhaps via localization to a non-nuclear compartment and/or interaction with components of other signalling pathways. However, the question of how VDRs are targetted to different cell types and how they are regulated remains unresolved. There have been many reports in the literature describing translational or transcriptional control of VDR levels, both homologously and heterologously, mostly in non-human systems.

A recent study (2) showed that in the kidney, alternative splicing of human VDR transcripts transcribed from a GC rich promotor generates several transcripts which vary only in their 5' UTRs. The present inventors have now identified further upstream exons of the VDR gene which generate 5' variant transcripts, suggesting that the expression of the VDR gene is regulated by more than one promoter. A subset of these transcripts is expressed in a restricted tissue-specific pattern and further variant transcripts have the potential to encode an N-terminally variant protein. These results may have implications for understanding the actions of 1,25-(OH)₂D₃ in different tissues and cell types, and the possibility that N-terminally variant VDR proteins may be produced has implications for altered activities such as transactivation function or subcellular localisation of the receptor protein. Furthermore, these variants, by their level, tissue specificity, subcellular localisation and functional activity, may yield targets for pharmaceutical intervention. The variants may also be useful in screening potential analogs and/or antagonists of vitamin D compounds.

Disclosure of the Invention:-

In a first aspect, the invention provides an isolated polynucleotide molecule encoding a human Vitamin D receptor (hVDR) isoform, said polynucleotide molecule comprising a nucleotide sequence which includes sequence that substantially corresponds or is functionally equivalent to that of exon 1d of the human VDR gene.

Exon 1d (referred to as exon 1b in the Australian Provisional Patent Specification No. PO9500) is a 96 bp exon located 296 bp downstream from exon 1a (2). The sequence of exon 1d is:

5'GTTTCCTTCTTCTGTCGGGGCGCCTTGGCATGGAGTGGAGGAATAAGAA
AAGGAGCGATTGGCTGTTCGATGGTGCTCAGAACTGCTGGAGTGGAGG3'
(SEQ ID NO: 1).

The nucleotide sequence of the polynucleotide molecule of the first aspect of the invention, preferably does not include sequence corresponding to that of exon 1a, exon 1f and/or exon 1e. However, the nucleotide sequence of the polynucleotide molecule of the first aspect of the invention, may or

may not include sequence that substantially corresponds or is functionally equivalent to that of exon 1b and/or exon 1c.

Preferably, the polynucleotide molecule of the first aspect comprises a nucleotide sequence which includes;

5 (i) sequence that substantially corresponds or is functionally equivalent to that of exons 1d, 1c and 2-9 and encodes a VDR isoform of approximately 477 amino acids,

(ii) sequence that substantially corresponds or is functionally equivalent to that of exons 1d and 2-9 and encodes a VDR isoform of
10 approximately 450 amino acids, or

(iii) sequence that substantially corresponds or is functionally equivalent to that of exons 1d and 2-9 and further includes a 152 bp intronic sequence, and encodes a truncated VDR isoform of approximately 72 amino acids.

15 Most preferably, the polynucleotide molecule of the first aspect of the invention comprises a nucleotide sequence substantially corresponding to that shown as SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4.

In a second aspect, the invention provides an isolated polynucleotide molecule encoding a human Vitamin D receptor (hVDR), said polynucleotide
20 molecule comprising a nucleotide sequence which includes sequence that substantially corresponds to that of exon 1f and/or 1c of the human VDR gene.

Exon 1f is a 207bp exon located more than 9kb upstream from exon 1a (2) bp upstream from exon 1c(8). The sequence of exon 1f is:

25

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5'TGCGACCTTGGCGGTGAGCCTGGGGACAGGGGTGAGGC
CAGAGACGGACGGACGCAGGGGCCCCGCCCCAAGGCGAGGG
AGAACAGCGGCACTAAGGCAGAAAGGAAGAGGGCGGTGTG
TTCACCCGCAGCCCCAATCCATCACTCAGCAACTCCTAGAC
30 GCTGGTAGAAAGTTCCTCCGAGGAGCCTGCCATCCAGTCGT
CCGTGCAG3' (SEQ ID NO: 5)
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Exon 1e is a 157 bp exon located 1826bp upstream from exon 1a (2).
The sequence of exon 1e is:

5'AGCCAGCATGAAACAGTGGGATGTGCAGAG
AGAAGATCTTGGGTCCAGTAGCTCTGACACTCCTCAGCTGT
AGAAACCTTGACAACCTCTGCACATCAGTTGTACAATGGAA
5 CGGTA'TTTTTACTCTTCATGTCTGAAAAGGCTATGATAA
AGATCAA3' (SEQ ID NO: 6)

The nucleotide sequence of the polynucleotide molecule of the second aspect of the invention, preferably does not include sequence corresponding to that of exon 1a, 1d or 1b. However, the nucleotide sequence of the polynucleotide molecule of the second aspect of the invention, may or may not include sequence that substantially corresponds or is functionally equivalent to that of exon 1c.

15 Preferably, the nucleotide molecule of the second aspect comprises a nucleotide sequence which includes sequence that substantially corresponds or is functionally equivalent to that of exons 1f and 2-9.

Most preferably, the polynucleotide molecule of the first aspect of the invention comprises a nucleotide sequence substantially corresponding to that shown as SEQ ID NO: 7.

20 The polynucleotide molecule of the first or second aspects may be incorporated into plasmids or expression vectors (including viral vectors), which may then be introduced into suitable host cells (e.g. bacterial, yeast, insect and mammalian host cells). Such host cells may be used to express the VDR or functionally equivalent fragment thereof encoded by the isolated polynucleotide molecule.

25 Accordingly, in a third aspect, the present invention provides a host cell transformed with the polynucleotide molecule of the first or second aspect.

30 In a fourth aspect, the present invention provides a method of producing a VDR or a functionally equivalent fragment thereof, comprising culturing the host cell of the first or second aspect under conditions enabling the expression of the polynucleotide molecule and, optionally, recovering the VDR or functionally equivalent fragment thereof.

35 Preferably, the host cell is of mammalian origin. Preferred examples include NIH 3T3 and COS 7 cells.

In a preferred embodiment, the VDR or functionally equivalent fragment thereof is localised to a cell membrane or other subcellular compartment as distinct from a nuclear localisation.

The polynucleotide molecules of the first aspect of the invention
5 encode novel VDR isoforms which may be of interest both clinically and commercially. By using the polynucleotide molecule of the present invention it is possible to obtain VDR isoform proteins or functionally equivalent fragments thereof in a substantially pure form.

Accordingly, in a fifth aspect, the present invention provides a human
10 VDR isoform or functionally equivalent fragment thereof encoded by a polynucleotide molecule of the first aspect, said VDR isoform or functionally equivalent fragment thereof being in a substantially pure form.

In a sixth aspect, the present invention provides an antibody or
15 antibody fragment capable of specifically binding to the VDR isoform of the fourth aspect.

The antibody may be monoclonal or polyclonal, however, it is presently preferred that the antibody is a monoclonal antibody. Suitable antibody fragments include Fab, F(ab')₂ and scFv.

In an eighth aspect, the present invention provides a non-human
20 animal transformed with a polynucleotide molecule according to the first or second aspect of the invention.

In a seventh aspect, the invention provides a method for detecting
agonist and/or antagonist compounds of a VDR isoform of the fourth aspect, comprising contacting said VDR isoform, functionally equivalent fragment
25 thereof or a cell transformed with and expressing the polynucleotide molecule of the first aspect, with a test compound under conditions enabling the activation of the VDR isoform or functionally equivalent fragment thereof, and detecting an increase or decrease in the activity of the VDR isoform or functionally equivalent fragment thereof.

30 An increase or decrease in activity of the receptor or functionally equivalent fragment thereof may be detected by measuring changes in interactions with known cofactors (e.g. SRC-1, GRIP-1 and TFIIB) or unknown cofactors (e.g. through use of the yeast dual hybrid system).

In a ninth aspect, the present invention provides an oligonucleotide or
35 polynucleotide probe comprising a nucleotide sequence of 10 or more nucleotides, the probe comprising a nucleotide sequence such that the probe

specifically hybridises to the polynucleotide molecule of the first or second aspect under high stringency conditions (Sambrook *et al.*, *Molecular Cloning: a laboratory manual*, Second Edition, Cold Spring Harbor Laboratory Press).

Preferably, the probe is labelled.

5 In a tenth aspect, the present invention provides an antisense polynucleotide molecule comprising a nucleotide sequence capable of specifically hybridising to an mRNA molecule which encodes a VDR encoded by the polynucleotide molecule of the first or second aspect, so as to prevent translation of the mRNA molecule.

10 Such antisense polynucleotide molecules may include a ribozyme region to catalytically inactivate mRNA to which it is hybridised.

The polynucleotide molecule of the first or second aspect of the invention may be a dominant negative mutant which encodes a gene product causing an altered phenotype by, for example, reducing or eliminating the
15 activity of endogenous VDR.

In an eleventh aspect, the invention provides an isolated polynucleotide molecule comprising a nucleotide sequence substantially corresponding or, at least, showing >75% (preferably >85% or, even more preferably, >95%) sequence identity to:

20

(i) 5'TGCGACCTTGCGGTTGAGCCTGGGGACAGGGGTGAGGCCAGAGACGGACGGACGCAGGGGCCCCGGCCCCAAGGCGAGGGAGAACAGCGGCACTAAGGCAGAAAGGAAGAGGGCGGTGTGTTTACCCGCAGCCCAATCCATCAC
25 TCAGCAACTCCTAGACGCTGGTAGAAAGTTCTTCGAGGAGCCTGCCATC
CAGTCGTGCGTGCAG 3'(exon 1f) (SEQ ID NO: 5).

(ii) 5'AGGCAGCATGAAACAGTGGGATGTGCAGAGAGAAGATCTGGGTC
CAGTAGCTCTGACACTCCTCAGCTGTAGAAACCTTGACAACTCTGCACAT
CAGTTGTACAATGGAACGGTATTTTTTACTCTTCATGTCTGAAAAGGCTA
30 TGATAAAGATCAA3' (exon 1e) (SEQ ID NO: 6), or

(iii) 5'GTTTCCTTCTTCTGTCCGGGCGCCTTGGCATGGAGTGGAGGAATA
AGAAAAGGAGCGATTGGCTGTGGATGGTGCTCAGAACTGCTGGAGTGA
GG3' (exon 1d) (SEQ ID NO: 1).

35

The polynucleotide molecules of the eleventh aspect may be useful as probes for the detection of VDR variant transcripts and as such may be useful in assessing cell or tissue-specific expression of variant transcripts.

The terms "substantially corresponds" and "substantially corresponding" as used herein in relation to nucleotide sequences is intended to encompass minor variations in the nucleotide sequence which due to degeneracy in the DNA code do not result in a substantial change in the encoded protein. Further, this term is intended to encompass other minor variations in the sequence which may be required to enhance expression in a particular system but in which the variations do not result in a decrease in biological activity of the encoded protein.

The term "functionally equivalent" as used herein in relation to nucleotide sequences encoding a VDR isoform is intended to encompass nucleotide sequence variants of up to 5% sequence divergence (i.e. retaining 95% or more sequence identity) which encode VDR isoforms of substantially equivalent biological activity(ies) as said VDR isoform.

The term "functionally equivalent fragment" as used herein in respect of a VDR isoform is intended to encompass functional peptide and polypeptide fragments of said VDR isoform which include the domain or domains which bestow the biological activity characteristic of said VDR isoform.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated step, component or feature or group of steps, components or features with or without the inclusion of a further step, component or feature or group of steps, components or features.

The invention will hereinafter be further described by way of the following non-limiting example and accompanying figures.

Brief description of the figures:-

FIG. 1. (A) Human VDR gene locus. Four overlapping cosmid clones were isolated from a human lymphocyte genomic library (Stratagene) and directly sequenced. Clone J5 extends from the 5' flanking region to intron 2; AE, from intron 1b to intron 5; D2, from intron 3 to the 3' UTR; WE, from intron 6 through the 3' flanking region. Sequence upstream of exon 1f was obtained by

anchored PCR from genomic DNA. (B) Structure of hVDR transcripts.

Transcripts 1–5 originate from exon 1a. Transcript 1 corresponds to the published cDNA (1). Transcripts 6–10 originate from exon 1d and transcripts 11–14 originate from exon 1f. Boxed numbers indicate the major transcript (based on the relative intensities of the multiple PCR products) within each exon-specific group of transcripts generated with a single primer set. While all transcripts have a translation initiation codon in exon 2, exon 1d transcripts have the potential to initiate translation upstream in exon 1d, with transcripts 6 and 9 encoding VDR proteins with extended N termini. (C) N-terminal variant proteins encoded by novel hVDR transcripts. Transcript 1 corresponds to the published cDNA sequence (1) and encodes the 427-aa hVDR protein. Transcripts 6 and 9 code for a protein with an extra 50 aa or 23 aa, respectively, at the N-terminal. The 23 aa of the hVDR A/B domain are shown in bold.

FIG. 2. RT-PCR analysis of expression of variant hVDR transcripts. (A) Exon 1a transcripts (220 bp, 301 bp, 342 bp, 372 bp, and 423 bp). (B) Exon 1d transcripts (224 bp, 305 bp, 346 bp, 376 bp, and 427 bp). (C) Exon 1f transcripts (228 bp, 309 bp, 387 bp, and 468 bp). RT-PCR was carried out with exon 1a-, 1d-, or 1f-specific forward primers and a common reverse primer in exon 3. The sizes of the PCR products and the pattern of bands are similar in A and B by virtue of the identical splicing pattern of exon 1a and 1d transcripts and the fact that primers were designed to generate PCR products of comparable sizes. All tissues and cell lines are human in origin.

FIG. 3. Functional analysis of sequence-flanking exons 1a and 1d (A) and exon 1f (B) in NIH 3T3 (solid bars) and COS 7 cells (open bars).

The parent vector pGL3basic was used as a promoterless control, and a promoter-chloramphenicol acetyltransferase (CAT) gene reporter construct was cotransfected as an internal control for transfection efficiency in each case. The activity of each construct was corrected for transfection efficiency and for the activity of the pGL3basic empty vector control and expressed as a percentage of the activity of the construct 1a(-488, +75) SEM of at least three separate transfections. Exon 1a and 1d flanking constructs are defined in relation to the transcription start site of exon

1a, designated 11, which lies 54 nt upstream of the published cDNA (1). Exon 1f flanking constructs are defined relative to the exon 1f transcription start site, designated 11. Transcription start sites were determined by the 5' termini of the longest RACE clones. The open box corresponds to the GC-rich region.

FIG 4. Provides the nucleotide sequence of novel exons detected by 5' RACE: (A) exon 1b (SEQ ID NO: 8), (B) exon 1f (SEQ ID NO: 5) [P1f is indicated by an arrow above the sequence], (C) exon 1e (SEQ ID NO: 6), (D) exon 1d (SEQ ID NO: 1) [in-frame ATG codons are highlighted and P1d is indicated by an arrow above the sequence]. Intronic sequences are shown in lower case. Canonical splice site consensus sequences are indicated in bold. The transcription start sites for exons 1f and 1d were determined by the 5' termini of RACE clones. No intron sequence is shown 3' to exon 1f as cosmid clone J5 terminated in the intron between exons 1f and 1e.

FIG 5. Provides the nucleotide sequence corresponding to transcript 6 (see figure 1) (SEQ ID NO: 2), together with the predicted amino acid sequence (SEQ ID NO: 9) of the encoded protein. Nucleotides 1-96 correspond to exon 1d; nucleotides 97-1463 correspond to exons 1c to the stop codon in exon 9 (or nucleotides -83-1283 of the hVDR cDNA (1)).

FIG 6. Provides the nucleotide sequence corresponding to transcript 9 (see figure 1) (SEQ ID NO: 3), together with the predicted amino acid sequence (SEQ ID NO: 10) of the encoded protein. Nucleotides 1-96 correspond to exon 1d; nucleotides 97 - 1382 correspond to exon 2 to the stop codon in exon 9 (or nucleotides -2 - 1283 of the hVDR cDNA (1)).

FIG 7. Provides the nucleotide sequence corresponding to transcript 10 (see figure 1) (SEQ ID NO: 4), together with the predicted amino acid sequence (SEQ ID NO: 11) of the encoded protein. Nucleotides 1-96 correspond to exon 1d; nucleotides 97-244 correspond to exon 2; nucleotides 245-396 correspond to intronic sequence immediately 3' to exon 2; nucleotides 397-1534 correspond to exons 3 to the stop codon in exon 9 (or nucleotides 146-1283 of the hVDR cDNA (1)).

FIG 8. Provides the nucleotide sequence corresponding to transcript 11 (see figure 1) (SEQ ID NO: 7), together with the predicted amino acid sequence (SEQ ID NO: 12) of the encoded protein. Nucleotides 1-207 correspond to exon 1f; nucleotides 208-1574 correspond to exon 1c to the stop codon in exon 9 (or nucleotides -83-1283 of the hVDR cDNA (1)).

Example:-

EXPERIMENTAL PROCEDURES

Isolation and Characterisation of Genomic Clones

A human lymphocyte cosmic library (Stratagene, La Jolla, Ca) was screened using a 2.1kb fragment of the hVDR cDNA encompassing the entire coding region but lacking the 3'UTR, a 241 bp PCR product spanning exons 1 to 3 of the human VDR cDNA, and a 303 bp PCR product spanning exons 3 and 4 of the hVDR cDNA. following standard colony hybridisation techniques. DNA probes were labelled by nick translation (Life Technologies, Gaithersburg, MD) with [α^{32} P] dCTP. Positively hybridising colonies were picked and secondary and tertiary screens carried out until complete purification. Cosmid DNA from positive clones was purified (Qiagen), digested with different restriction enzymes and characterised by Southern blot analysis using specific [γ^{32} P]ATP labelled oligonucleotides as probes. Cosmid clones were directly sequenced using dye-termination chemistry and automated fluorescent sequencing on an ABI Prism. 377 DNA Sequencer (Perkin-Elmer, Foster City, Ca). Sequence upstream of the most 5' cosmid was obtained by anchored PCR from genomic DNA using commercially available anchor ligated DNA (Clontech, Palo Alto, Ca).

Rapid Amplification of cDNA 5-prime Ends (5'-RACE)

Alternative 5' variants of the human VDR gene were identified by 5'RACE using commercially prepared anchor-ligated cDNA (Clontech) following the instructions of the manufacturer. Two rounds of PCR using nested reverse primers in exons 3 and 2 (P 1: 5'ccgcttcattgcttcgcctgaagaagcc-3', P2: 5'-tgcagaattcacaggtcatagcattgaag-3') were carried out on a Corbett FTS-4000 Capillary Thermal Sequencer (Corbett Research, NSW, Australia). After 26 cycles of PCR, 2% of the primary reaction was reamplified for 31 cycles.

The PCR products were cloned into PUC18 and sequenced by the dideoxy chain termination method.

Cell-Culture

- 5 The embryonal kidney cell line, HEK-293, an embryonic intestine cell line, Intestine-407 and WS 1, a foetal skin fibroblast cell line were all cultured in Eagle's MEM with Earle's BSS and supplemented with either 10% heat-inactivated FBS, 15% FBS or 10% FBS with non-essential amino acids, respectively. The osteosarcoma cell lines MG-63 and Saos-2 were cultured in
10 Eagle's MEM with nonessential amino acids and 10% heat-inactivated FBS and McCoy's 5a medium with 15% FBS, respectively. The breast carcinoma cell line T47D and the colon carcinoma cell lines LIM 1863 and COLO 206F were cultured in RPMI medium supplemented with 0.2 IU bovine insulin/ml and 10% FBS, 5% FBS or 10% FBS, respectively. LIM 1863 were a gift from
15 R.H. Whitehead (3). IIK-2 kidney proximal tubule cells were grown in keratinocyte-serum free medium supplemented with 5ng/ml recombinant EGF, 40ug/ml bovine pituitary extract. BC1 foetal osteoblast-like cells were kindly donated by R. Mason (4) and were grown in Eagle's MEM with 5% FBS and 5mg/L vitamin C. Unless otherwise stated all cell lines were obtained
20 from the American Type Culture Collection (Manassas, VA).

Reverse Transcriptase-PCR (RT-PCR).

- Total RNA extracted from approximately 1.5×10^6 cells, from leukocytes prepared from 40 ml blood, or from human tissue using acid-
25 phenol extraction was purified by using a guanidium isothiocyanate-caesium chloride step gradient. First-strand cDNA was synthesized from 5 µg of total RNA primed with random hexamers (Promega) using Superscript II reverse transcriptase (Life Technologies). One-tenth of the cDNA (2µl) was used for subsequent PCR, with 36 cycles of amplification, using exon-specific forward
30 primers (exon 1a: corresponding to nucleotides 1-21 of hVDR cDNA (1); exon 1d: 5'-GGCTGTCGATGGTGCTCAGAAC-3'; exon 1f: 5'-AAGTTCCTCCGAGGAGCCTGCC-3'); and a common reverse primer in exon 3 [corresponding to nucleotides 301-280 of hVDR cDNA (1)]. All RT-PCRs were repeated multiple times by using
35 RNA/cDNA prepared at different times from multiple sources. Each PCR included an appropriate cDNA-negative control, and additional controls

included RT-negative controls prepared alongside cDNA and RNA/cDNA prepared from VDR-negative cell lines. PCR products were separated on 2% agarose and visualized with ethidium bromide staining.

5 *Functional Analysis of hVDR Gene Promoters.*

Sequences flanking exons 1a, 1d, and 1f (see Fig. 1A) were PCR-amplified by using Pfu polymerase (Stratagene) and cloned into the pGL3basic vector (Promega) upstream of the luciferase gene reporter. Promoter-reporter constructs were transfected into NIH 3T3 and COS 7 cells
10 by using the standard calcium phosphate-precipitation method. Cells were seeded at $2.3 \pm 2.5 \times 10^6$ per 150-cm² flask the day before transfection. Several hours before the precipitates were added the medium was changed to DMEM with 2% charcoal-stripped FBS. Cells were exposed to precipitate for 16 h before subculturing and were harvested 24 h later. The parent vector
15 pGL3basic was used as a promoterless control in these experiments and a simian virus 40 promoter-chloramphenicol acetyltransferase (CAT) gene reporter construct was cotransfected as an internal control for transfection efficiency in each case. The activity of each construct was corrected for transfection efficiency and for the activity of the pGL3 basic empty vector
20 control and expressed as a percentage of the activity of the construct 1a(-488, +75). Luciferase and CAT assays were carried out in triplicate, and each construct was tested in transfection at least three times.

25 *RESULTS*

Identification of Alternative 5' Variants of the hVDR Gene.

Upstream exons were identified in human kidney VDR transcripts by 5' RACE (exons 1f, 1e, 1d, and 1b) and localized by sequencing of cosmid clones (Fig. 1A). To verify these results and to characterize the structure of
30 the 5' end of the VDR gene, exon-specific forward primers were used with a common reverse primer in exon 3 to amplify specific VDR transcripts from human tissue and cell line RNA (Fig. 1B). The identity of these PCR products was verified by Southern blot and by cloning and sequencing. Five different VDR transcripts originating from exon 1a were identified. The major
35 transcript (transcript 1 in Fig. 1B) corresponds to the published cDNA sequence (1). Three less-abundant forms (2, 3, and 4 in Fig. 1B) arise from

alternative splicing of exon 1c and a novel 122-bp exon 1b into or out of the final transcript. These three variant transcripts were described recently by Pike and colleagues (2). A fifth minor variant was identified (5 in Fig. 1B) that lacks exons 1b and 1c, but includes an extra 152 bp of intronic sequence immediately 3' to exon 2, potentially encoding a truncated protein as a result of an in-frame termination codon in intron 2.

Four more transcripts were characterized that originate from exon 1f, a novel 207-bp exon more than 9 kb upstream from exon 1a. The major 1f-containing transcript (11 in Fig. 1B) consists of exon 1f spliced immediately adjacent to exon 1c. Three less-abundant variants (12, 13, and 14 in Fig. 1B) arise from alternative splicing of exon 1c and a novel 159-bp exon 1e into or out of the final transcript. All these hVDR variants differ only in their 5' UTRs and encode identical proteins from translation initiation in exon 2.

Of considerable interest, another five hVDR transcripts were identified that originate from exon 1d, a novel 96-bp exon located 296 bp downstream from exon 1a. The major exon 1d-containing transcript (6 in Fig. 1B) utilizes exon 1d in place of exon 1a of the hVDR cDNA. Three minor variants (7, 8, and 9 in Fig. 1B) arise from alternative splicing of exons 1b and 1c into or out of the transcript, analogous to the exon 1a-containing variants 2, 3, and 4. A fifth minor variant transcript (10 in Fig. 1B) lacks exons 1b and 1c, but includes 152 bp of intron 2 analogous to the exon 1a-containing transcript 5, and also potentially encodes a truncated protein. Two of these exon 1d-containing hVDR transcripts encode an N-terminal variant form of the hVDR protein. Utilization of an ATG codon in exon 1d, which is in a favorable context and in-frame with the major translation start site in exon 2, would generate a protein with an additional 50 aa N-terminal to the ATG codon in exon 2 in the case of variant 6 or 23 aa in the case of variant 9 (Fig. 1C).

The relative level of expression of the different transcripts is difficult to address with PCR since relatively minor transcripts may be amplified.

However, Southern blots of PCR products from the linear range of PCR amplification indicated that equivalent amounts of PCR product were accumulated after 26 cycles for exon 1a transcripts compared with 30 cycles for exon 1d transcripts, suggesting that 1d abundance is about 5% of that of 1a transcripts. This is consistent with the frequency of clones selected and sequenced from RACE analysis of two separate samples of kidney RNA: 1a (21/27; 78%), 1d (2/27; 7%), and 1f (4/27; 15%). RT-PCR with exon 1a-, 1d-, or

1f-specific forward primers and reverse primers in exons 7 or 9, followed by cloning and sequencing, suggests that these 5' variant transcripts are not associated with differences at the 3' end of the transcript.

5 *Exon-Intron Organization of the hVDR Gene.*

Overlapping cosmid clones were isolated from a human lymphocyte genomic library and characterized by hybridization to exon-specific oligonucleotide probes (Fig. 1A). The exon-intron boundaries of the hVDR gene were determined by comparison of the genomic sequence from cosmid clones with the cDNA sequence. Upstream exons were localized in the VDR gene by sequencing cosmid clones, which extend approximately 7 kb into the intron between exons 1e and 1f, enabling verification of both their sequence and the presence of consensus splice donor/acceptor sites. Sequence upstream of exon 1f was obtained by anchored PCR from genomic DNA by using commercially available anchor-ligated DNA (CLONTECH). In total, the hVDR gene spans more than 60 kb and consists of at least 14 exons (Fig. 1A).

Tissue-Specific Expression of hVDR Transcripts.

The pattern of expression of variant hVDR transcripts was examined by RT-PCR in a variety of cell lines and tissues with exon 1a-, 1d-, or 1f-specific forward primers and a common reverse primer in exon 3. Exon 1a and 1d transcripts (Fig. 1B, variants 1-10) were coordinately expressed in all RNA samples analyzed (Fig. 2A and B). Exon 1f transcripts (Fig. 1B, variants 11-14), however, were detected only in RNA from human kidney tissue (two separate samples), human parathyroid adenoma tissue, and an intestinal carcinoma cell line, LIM 1863 (Fig. 2C). Interestingly, these represent major target tissues for the calcitropic effects of vitamin D

Functional Analysis of hVDR Gene Promoters.

Promoter activities of the 5' flanking regions of exons 1a, 1d, and 1f were examined in NIH 3T3 and COS 7 cells (Fig. 3). Sequences flanking exon 1a exhibited high promoter activity in both cell lines (Fig. 3A). Maximum luciferase expression of 36- and 54-fold over the empty vector was attained for construct 1a(-488, +75) in NIH 3T3 and COS 7 cells, respectively. This activity could be attributed largely to a GC-rich region containing multiple consensus Sp1-binding motifs lying within 100 bp immediately adjacent to

the transcription start site. This region alone, upstream of a luciferase reporter [construct 1a(-94, +75)], accounted for 43% of the maximum activity observed in NIH 3T3 cells and 86% of the maximum observed in COS 7 cells. The removal of this GC-rich region [construct 1a(-29, +75)] reduced luciferase activity to only 13% of the maximum in NIH 3T3 and 19% in COS 7 cells. Despite the fact that VDR transcripts that originated from exon 1d were identified, distinct promoter activity was not associated with sequences within 300 bp of exon 1d [constructs 1d(+87, +424) and 1d(+244, +424)]; rather, the sequence immediately adjacent to exon 1d may contain a suppressor element (Fig. 3A). Construct 1a-1d(-846, +470), spanning the 5' flanking regions of both exons 1a and 1d, resulted in only 42% and 60% of the activity of 1a(-898, +75) in NIH 3T3 and COS 7 cells, whereas the 3' deletion of 227 bp restored luciferase activity to 65% and 97% of the activity of 1a(-898, +75), respectively. Similarly, the 5' truncated construct 1a-1d (-94, +470), spanning the 5' flanking regions of both 1a and 1d, resulted in only 35% and 40% of the activity of 1a(-94, +75), while a further 3' deletion of 227 bp restored luciferase activity to 69% and 91% of the activity of 1a(-94, +75) in NIH 3T3 and COS 7 cells. It is possible that transcription from exons 1a and 1d is driven by overlapping promoter regions rather than from two distinct promoters, as has been described for the mouse androgen receptor gene.

Sequence upstream of exon 1f showed significant promoter activity in NIH 3T3 cells of 22% of that of the most active construct, 1a(-488, +75), or 9-fold over pGL3basic [construct 1f(-1168, +58)] (Fig. 3B). A shorter construct [1f(-172, +58)] had similar activity, with evidence of a suppressor element (between nucleotides -278 and +172) able to repress luciferase activity by 70%. Interestingly, the same constructs were not active in COS 7 cells. This cell line-specific activity of exon 1f flanking sequences may reflect a requirement for tissue- or cell-specific protein factors.

Identification of VDR isoforms in whole cell lysates

The existence of a VDR isoform including exons 1d and 1c has been confirmed in cell lysates from multiple human, monkey, rat and mouse cell lines derived from kidney, intestine, liver and bone, by immunoprecipitation (using the anti-VDR 9A7 rat monoclonal antibody; Affinity Bioreagents Inc.,

Golden, Colorado) followed by Western blot analysis. The 1d- and 1c-exon-specific antibodies detected the same band in all immunoprecipitations.

DISCUSSION

5

The present inventors have identified 5' variant transcripts of the hVDR that suggest the existence of alternative promoters. These transcripts may not have been discriminated in previous Northern analyses because of their similarity in size. Transcription initiation from exons 1a or 1f and
10 alternative splicing generate VDR transcripts that vary in their 5' UTRs but encode the same 427-aa protein. Transcription initiation from exon 1d and alternative splicing generate hVDR transcripts with the potential to encode variant proteins with an additional 50 or 23 aa at the N terminus. There was
15 no evidence that these 5' variants are associated with differences at the 3' end of the transcript. Although isoforms are common in other members of the nuclear receptor superfamily, the only evidence for isoforms of the hVDR is a common polymorphism in the triplet encoding the initiating methionine of the 427-aa form of the VDR that results in initiation of translation at an
20 alternative start codon beginning at the 10th nucleotide down-stream, encoding a protein truncated by 3 aa at the N terminus (5). Similarly, two forms of the avian VDR, differing in size by 14 aa, are generated from a single transcript by alternative translation initiation (6), and in the rat a dominant-negative VDR is generated by intron retention (7).

Heterogeneity in the 5' region is a common feature of other nuclear
25 receptor genes. Tissue-specific alternative-promoter usage generates multiple transcripts of the human estrogen receptor α (ER α), the human and rat mineralocorticoid receptors, and the mouse glucocorticoid receptor (GR), which differ in their 5' UTRs but code for identical proteins. However, other
30 members of the nuclear receptor superfamily have multiple, functionally distinct isoforms arising from differential promoter usage and/or alternative splicing. The generation of N-terminal variant protein isoforms has been described for the progesterone receptor (PR), peroxisome proliferator-activated receptor (PPAR α), and the retinoid and thyroid receptors. Some
35 receptor isoforms exhibit differential promoter-specific transactivation activity. The N-terminal A/B regions of many nuclear receptor proteins possess a ligand-independent transactivation function (AF1). An AF1

domain has been demonstrated for the thyroid receptor b1 (TRb1), ER, GR, PR, PPAR γ , and the retinoid receptors. The activity of the AF1 domain has been shown to vary in both a tissue- and promoter-specific manner. The N-terminal A/B region of nuclear receptors is the least-conserved domain across the family and between receptor subtypes, varying considerably both in length and sequence. The VDR, however, is unusual as its N-terminal A/B region is much shorter than that of other nuclear receptors, with only 23 aa N-terminal to the DNA-binding domain, and deletion of these residues seems to have no effect on VDR function. This region in other receptors is associated with optimal ligand-dependent transactivation and can interact directly with components of the basal transcription complex. Two stretches of basic amino acid residues, RNKKR and RPHRR, in the predicted amino acid sequences of the variant hVDR N termini (Fig. 1C) resemble nuclear localization signals. An N-terminal variant VDR protein therefore might exhibit different transactivation potential, possibly mediated by different protein interactions, or may specify a different subcellular localization. The tissue-specific expression of exon 1f-containing transcripts is mediated by a distal promoter more than 9 kb upstream of exons 1a and 1d. Exon 1f transcripts were detected only in kidney tissue, parathyroid adenoma tissue, and an intestinal cell line, LIM 1863. It is interesting that these tissues represent major target tissues for the calcitropic effects of vitamin D. The absence of 1f-containing transcripts in two other kidney cell lines, HK-2 (proximal tubule) and HEK-293 (embryonal kidney), as well as one other embryonal intestinal cell line, Intestine-407, suggests that the expression of 1f transcripts is cell type-specific. The cell line-specific activity of exon 1f flanking sequences in promoter reporter assays may reflect a requirement for tissue- or cell-specific protein factors to mediate expression from this promoter.

This study has demonstrated that expression of the human VDR gene, which spans more than 60 kb and consists of 14 exons, is under complex transcriptional control by multiple promoters. The expression of multiple exon 1f transcripts is mediated by utilization of a distal tissue-specific promoter. Transcription from a proximal promoter, or promoters, generates multiple variant hVDR transcripts, two of which code for N-terminal variant proteins. Multiple, functionally distinct isoforms mediate the tissue- and/or developmental-specific effects of many members of the nuclear receptor

superfamily. Although the actual relative abundance of the various transcripts and their levels of translation *in vivo* have not yet been characterized, the results suggest that major variant isoforms of the hVDR exist. Differential regulation of these hVDR gene promoters and of
5 alternative splicing of variant VDR transcripts may have implications for understanding the various actions of $1,25\text{-(OH)}_2\text{D}_3$ in different cell types, and variant VDR transcripts may play a role in tissue specific VDR actions in bone and calcium homeostasis.

10

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to
15 be considered in all respects as illustrative and not restrictive.

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Title of the Invention: Isoforms of the Human Vitamin D Receptor

• 140 •

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11605-12

(170) PatentIn Ver. 2.0

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 195 200 205

Glu Glu Asp Ser Asp Asp Pro Ser Val Thr Leu Glu Leu Ser Gln Leu
 210 215 220

Ser Met Leu Pro His Leu Ala Asp Leu Val Ser Tyr Ser Ile Gln Lys
 225 230 235 240

Val Ile Gly Phe Ala Lys Met Ile Pro Gly Phe Arg Asp Leu Thr Ser
 245 250 255

Glu Asp Gln Ile Val Leu Leu Lys Ser Ser Ala Ile Glu Val Ile Met
 260 265 270

Leu Arg Ser Asn Glu Ser Phe Thr Met Asp Asp Met Ser Trp Thr Cys
 275 280 285

28

Gly Asn Gln Asp Tyr Lys Tyr Arg Val Ser Asp Val Thr Lys Ala Gly
 290 295 300
 His Ser Leu Glu Leu Ile Glu Pro Leu Ile Lys Phe Gln Val Gly Leu
 305 310 315 320
 Lys Lys Leu Asn Leu His Glu Gln Glu His Val Leu Leu Met Ala Ile
 325 330 335
 Cys Ile Val Ser Pro Asp Arg Pro Gly Val Gln Asp Ala Ala Leu Ile
 340 345 350
 Glu Ala Ile Gln Asp Arg Leu Ser Asn Thr Leu Gln Thr Tyr Ile Arg
 355 360 365
 Cys Arg His Pro Pro Pro Gly Ser His Leu Leu Tyr Ala Lys Met Ile
 370 375 380
 Gln Lys Leu Ala Asp Leu Arg Ser Leu Asn Glu Gln His Ser Lys Gln
 385 390 395 400
 Tyr Arg Cys Leu Ser Phe Gln Pro Glu Cys Ser Met Lys Leu Thr Pro
 405 410 415
 Leu Val Leu Glu Val Phe Gly Asn Glu Ile Ser
 420 425

Claims:-

1. An isolated polynucleotide molecule encoding a human Vitamin D receptor (hVDR) isoform, said polynucleotide molecule comprising a
5 nucleotide sequence which includes sequence that substantially corresponds or is functionally equivalent to that of exon 1d of the human VDR gene.
2. A polynucleotide molecule according to claim 1, wherein said
10 nucleotide sequence further includes sequence that substantially corresponds or is functionally equivalent to that of exon 1b and/or exon 1c.
3. A polynucleotide molecule according to claim 1, wherein the nucleotide sequence includes:
 - (i) sequence that substantially corresponds or is functionally
15 equivalent to that of exons 1d, 1c and 2-9 and encodes a VDR isoform of approximately 477 amino acids,
 - (ii) sequence that substantially corresponds or is functionally equivalent to that of exons 1d and 2-9 and encodes a VDR isoform of approximately 450 amino acids, or
 - 20 (iii) sequence that substantially corresponds or is functionally equivalent to that of exons 1d and 2-9 and further includes a 152bp intronic sequence and encodes a truncated VDR isoform of approximately 72 amino acids.
- 25 4. A polynucleotide molecule according to claim 1, wherein the nucleotide sequence substantially corresponds to that shown as SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4.
5. An isolated polynucleotide molecule encoding a human Vitamin D
30 receptor (hVDR), said polynucleotide molecule comprising a nucleotide sequence which includes sequence that substantially corresponds or is functionally equivalent to that of exon 1f and/or 1e of the human VDR gene.
6. A polynucleotide molecule according to claim 5, wherein the
35 nucleotide sequence further includes sequence that substantially corresponds or is functionally equivalent to that of exon 1c.

7 A polynucleotide molecule according to claim 5, wherein the
nucleotide sequence includes sequence that substantially corresponds or is
functionally equivalent to that of exons 1f and 2-9.

5 8. A polynucleotide molecule according to claim 5, wherein the
nucleotide sequence substantially corresponds to that shown as SEQ ID NO:
7.

10 9. A plasmid or expression vector including a polynucleotide molecule
according to any one of the preceding claims.

10. A host cell transformed with a polynucleotide molecule according to
any one of claims 1-8 or a plasmid or expression vector according to claim 9.

15 11. A host cell according to claim 10, wherein the cell is a mammalian
cell.

20 12. A host cell according to claim 10, wherein the cell is a NIH 3T3 or COS
7 cell.

25 13. A method of producing a VDR or VDR isoform or functionally
equivalent fragments thereof, comprising culturing a host cell of any one of
claims 10-12 under conditions enabling the expression of the polynucleotide
molecule and, optionally, recovering the VDR or VDR isoform or functionally
equivalent fragments thereof.

30 14. A method according to claim 13, wherein the VDR or VDR isoform or
functionally equivalent fragments thereof are expressed onto the host cell
membrane or other sub-cellular compartment.

35 15. A human Vitamin D receptor (hVDR) isoform or functionally
equivalent fragment thereof encoded by a polynucleotide molecule according
to any one of claims 1-4, said hVDR isoform or functionally equivalent
fragment thereof being in a substantially pure form

16. An antibody or antibody fragment capable of specifically binding to a VDR isoform according to claim 15.

17. A non-human animal transformed with a polynucleotide molecule according to any one of claims 1-8.

18. A method for detecting agonist and/or antagonist compounds of a VDR isoform of claim 15, comprising contacting said VDR isoform, functionally equivalent fragment thereof or a cell transformed with and expressing a polynucleotide molecule according to any one of claims 1-4, with a test compound under conditions enabling the activation of the VDR isoform or functionally equivalent fragment thereof, and detecting an increase or decrease in the activity of the VDR isoform or functionally equivalent fragment thereof.

19. An oligonucleotide or polynucleotide probe comprising a nucleotide sequence of 10 or more nucleotides, the probe comprising a nucleotide sequence such that the probe specifically hybridises to a polynucleotide molecule according to any one of claims 1-8 under high stringency conditions.

20. An antisense polynucleotide molecule comprising a nucleotide sequence capable of specifically hybridising to a mRNA molecule which encodes a VDR or VDR isoform encoded by a polynucleotide molecule according to any one of claims 1-8, so as to prevent translation of the mRNA molecule.

21. An isolated polynucleotide molecule comprising a nucleotide sequence showing greater than 75% sequence identity to:

(i) 5'TGCGACCTTGGCGGTGAGCCTGGGGACAGGGGTGAGGCCAGAGACGGACGGACGCAGGGGCCCCGGCCCAAGGCGAGGGAGAACAGCGGCACTAAGGCAGAAAGGAAGAGGGCGGTGTGTTACCCGCAGCCCAATCCATCAC TCAGCAACTCCTAGACGCTGGTAGAAAGTTCCCTCCGAGGAGCCTGCCATC CAGTCGTGCGTGCAG3' (SEQ ID NO: 5)

(ii) 5'AGGCAGCATGAAACAGTGGGATGTGCAGAGAGAAGATCTGGGTC
CAGTAGCTCTGACACTCCTCAGCTGTAGAAACCITGACAACTCTGCACAT
CAGTTGTACAATGGAACGGTATTTTTTACTCTTCATGTCTGAAAAGGCTA
TGATAAAGATCAA3' (SEQ ID NO: 6), or

5

(iii) 5'GTTTCCTTCTTCTGTGCGGGGCGCCTTGGCATGGAGTGGAGGAATA
AGAAAAGGAGCGATTGGCTGTCCATGGTGCTCAGAACTGCTGGAGTGGA
GG3' (SEQ ID NO: 1)

10 22. An isolated polynucleotide molecule comprising a nucleotide sequence
showing greater than 85% sequence identity to:

(i) 5'TGCGACCTTGGCGGTGAGCCTGGGGACAGGGGTGAGGCCAGAGA
CGGACGGACGCAGGGGCCCCGGCCCAAGGCGAGGGAGAAACAGCGGCACTA
15 AGGCAGAAAGGAAGAGGGCGGTGTGTTACCCCGCAGCCCAATCCATCAC
TCAGCAACTCCTAGACGCTGGTAGAAAGTTCCTCCGAGGAGCCTGCCATC
CAGTCGTGCGTGCCAG3' (SEQ ID NO: 5)

(ii) 5'AGGCAGCATGAAACAGTGGGATGTGCAGAGAGAAGATCTGGGTC
20 CAGTAGCTCTGACACTCCTCAGCTGTAGAAACCTTGACAACTCTGCACAT
CAGTTGTACAATGGAACGGTATTTTTTACTCTTCATGTCTGAAAAGGCTA
TGATAAAGATCAA3' (SEQ ID NO: 6), or

(iii) 5'GTTTCCTTCTTCTGTGCGGGGCGCCTTGGCATGGAGTGGAGGAATA
25 AGAAAAGGAGCGATTGGCTGTCCATGGTGCTCAGAACTGCTGGAGTGGA
GG3' (SEQ ID NO: 1).

30 23. An isolated polynucleotide molecule comprising a nucleotide sequence
showing greater than 95% sequence identity to:

(i) 5'TGCGACCTTGGCGGTGAGCCTGGGGACAGGGGTGAGGCCAGAGA
CGGACGGACGCAGGGGCCCCGGCCCAAGGCGAGGGAGAAACAGCGGCACTA
AGGCAGAAAGGAAGAGGGCGGTGTGTTACCCCGCAGCCCAATCCATCAC
35 TCAGCAACTCCTAGACGCTGGTAGAAAGTTCCTCCGAGGAGCCTGCCATC
CAGTCGTGCGTGCCAG3' (SEQ ID NO: 5)

(ii) 5'AGGCAGCATGAAACAGTGGGATGTGCAGAGAGAAGATCTGGGTC
CAGTAGCTCTGACACTCCTCAGCTGTAGAAACCTTGACAACTCTGCACAT
CAGTTGTACAATGGAACGGTATTTTTTACTCTTCATGTCTGAAAAGGCTA
TGATAAAGATCAA3' (SEQ ID NO: 6), or

5

(iii) 5'GTTTCCTTCTTCTGTTCGGGGCGCCTTGGCATGGAGTGGAGGAATA
AGAAAAGGAGCGATTGGCTGTTCGATGGTGCTCAGAACTGCTGGAGTGGA
GG3' (SEQ ID NO: 1)

10

24. An isolated polynucleotide molecule comprising nucleotide sequence
substantially corresponding to:

15

(i) 5'TGCGACCTTGGCGGTGAGCCTGGGGACAGGGGTGAGGCCAGAGA
CGGACGGACGCAGGGGCCCCGGCCCAAGGCGAGGGAGAACAGCGGCACTA
AGGCAGAAAGGAAGAGGGCGGTGTGTTCACCCGAGCCCAATCCATCAC
TCAGCAACTCCTAGACGCTGGTAGAAAGTTCCTCCGAGGAGCCTGCCATC
CAGTCGTGCGTGCAG3' (SEQ ID NO: 5)

20

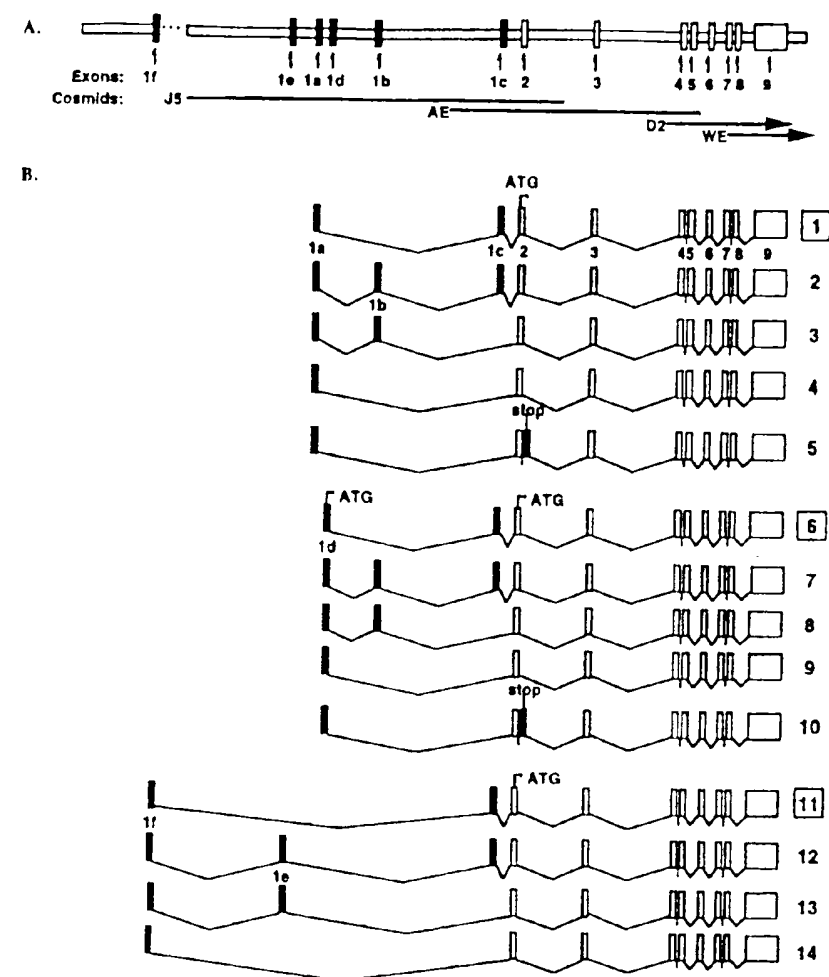
(ii) 5'AGGCAGCATGAAACAGTGGGATGTGCAGAGAGAAGATCTGGGTC
CAGTAGCTCTGACACTCCTCAGCTGTAGAAACCTTGACAACTCTGCACAT
CAGTTGTACAATGGAACGGTATTTTTTACTCTTCATGTCTGAAAAGGCTA
TGATAAAGATCAA3' (SEQ ID NO: 6), or

25

(iii) 5'GTTTCCTTCTTCTGTTCGGGGCGCCTTGGCATGGAGTGGAGGAATA
AGAAAAGGAGCGATTGGCTGTTCGATGGTGCTCAGAACTGCTGGAGTGGA
GG3' (SEQ ID NO: 1)

30

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Transcript 1:	MEAMA ASTSL PDPGD FDRNV PRI	DBD	427aa
Transcript 6:	MEWRN KKRSD WLSMY LRTAG VEEAF GSEVS VRPIR RAPLG STYLP PAPSG	MEAMA ASTSL PDPGD FDRNV PRI	DBD 477aa
Transcript 9:	MEW RNKKR SDWLS MVLRT AGVEG	MEAMA ASTSL PDPGD FDRNV PRI	DBD 450aa

FIGURE 1

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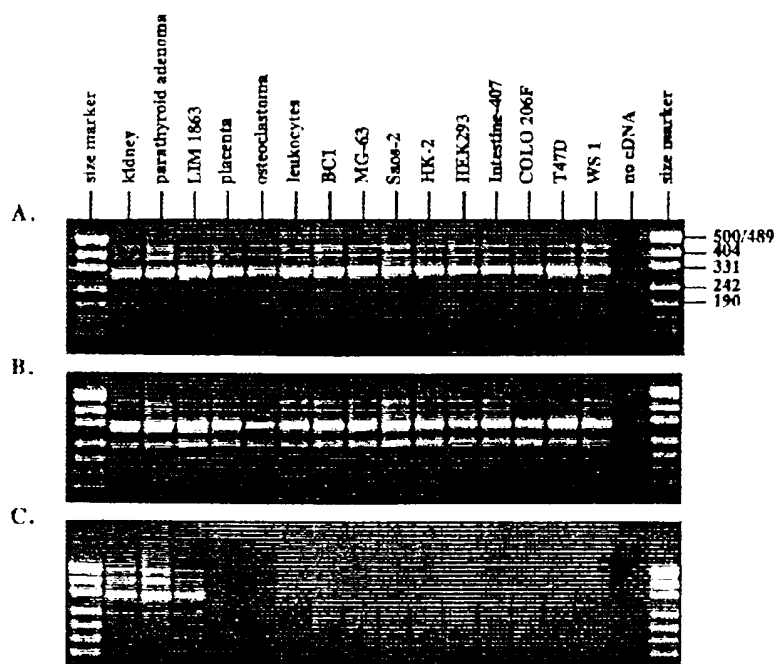


FIGURE 2

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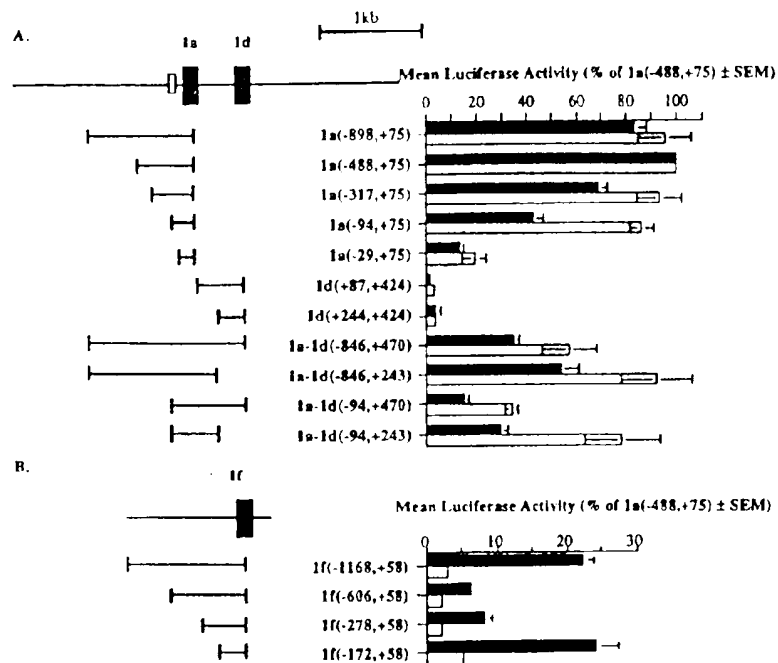


FIGURE 3

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- A. 5'...atcccttaag GGCTCCTGAACCTAGCCCAGCTGGACGGAG
AAATGGACTCTAGCCTCCTCTGATAGCCTCATGCCAGGCC
CGTGCACATTGCTTTGCTTGCCTCCCTCAATCCTCATAGCT
TCTCTTTGGGgtaagtacag...3'
- B. 5'...TGCGACCTTGGCGGTGAGCCTGGGGACAGGGGTGAGGC
CAGAGACGGACGGACGAGGGGCCCCGGCCCAAGGCGAGGG
AGAACAGCGGCACTAAGGCAGAAAGGAAGAGGGCGGTGTG
TTCACCCGCAGCCCAATCCATCACTCAGCAACTCCTAGAC
GCTGGTAGAAAGTTCTCCGAGGAGCCTGCCATCCAGTCGT
GCGTGCAG...3'
- C. 5'...tgtttttag AGGCAGCATGAAACAGTGGGATGTGCAGAG
AGAAGATCTGGGTCCAGTAGCTCTGACACTCCTCAGCTGT
AGAAACCTTGACAACTCTGCACATCAGTTGTACAATGGAA
CGGTATTTTTTACTCTTCATGTCTGAAAAGGCTATGATAA
AGATCAAgtaatatt...3'
- D. 5'...GTTTCCTTCTTCTGTCGGGGCGCCTTGGCAGGAGTGG
AGGAATAAGAAAAGGAGCGATTGGCTGTCCGATGCTGCTCA
GAACTGCTGGAGTGGAGGgtgtgtaacc...3'

FIGURE 4

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FIGURE 5 TRANSCRIPT 6

(Sequence Range: 1 to 1463)

```

      10      20      30      40      50
      *      *      *      *      *
GTTTCCTTCT TCTGTCGGGG CGCCTTGGCA TGGAGTGGAG GAATAAGAAA
CAAAGGAAGA AGACAGCCCC GCGGAACCGT ACCTCACCTC CTTATTCTTT
                               MetGluTrpArg AsnLysLys>

      60      70      80      90     100
      *      *      *      *      *
AGGAGCGATT GGCTGTCGAT GGTGCTCAGA ACTGCTGGAG TGGAGGAAGC
TCCTCGCTAA CCGACAGCTA CCACGAGTCT TGACGACCTC ACCTCCTTCG
ArgSerAsp TrpLeuSerMet ValLeuArg ThrAlaGly ValGluGluAla>

     110     120     130     140     150
      *      *      *      *      *
CTTTGGGTCT GAAGTGCTCTG TGAGACCTCA CAGAAGAGCA CCCCTGGGCT
GAAACCCAGA CTTACAGAC ACTCTGGAGT GTCTTCTCGT GGGGACCCGA
PheGlySer GluValSer ValArgProHis ArgArgAla ProLeuGly>

     160     170     180     190     200
      *      *      *      *      *
CCACTTACCT GCCCCCTGCT CCTTCAGGGA TGGAGGCAAT GGGGGCCAGC
GGTGAATGGA CGGGGGACGA GGAAGTCCCT ACCTCGTTA CCGCCGGTGG
SerThrTyrLeu ProProAla ProSerGly MetGluAlaMet AlaAlaSer>

     210     220     230     240     250
      *      *      *      *      *
ACTTCCCTGC CTGACCCTGG AGACTTTGAC CGGAAGGTGC CCGGATCTG
TGAAGGGACG GACTGGGACC TCTGAACTG GCCTTGCACG GGGCTTAGAC
ThrSerLeu ProAspProGly AspPheAsp ArgAsnVal ProArgIleCys>

     260     270     280     290     300
      *      *      *      *      *
TGGGGTGTGT GGAGACCGAG CCACTGGCTT TCACTTCAAT GCTATGACCT
ACCCACACA CCTCTGGCTC GGTGACCGAA AGTGAAGTTA CGATACTGGA
GlyValCys GlyAspArg AlaThrGlyPhe HisPheAsn AlaMetThr>

     310     320     330     340     350
      *      *      *      *      *
GTGAAGGCTG CAAAGGCTTC TTCAGGCGAA GCATGAAGCG GAAGGCACTA
CACTTCCGAC GTTTCGGAAG AAGTCCGCTT CGTACTTCGC CTCCGTGAT
CysGluGlyCys LysGlyPhe PheArgArg SerMetLysArg LysAlaLeu>

     360     370     380     390     400
      *      *      *      *      *
TTCACCTGCC CCTTCAACGG GGACTTCCGC ATCAACAAGG ACAACCGACG
AAGTGGACGG GGAAGTTGCC CCTGAGGCG TAGTGGTTC TGTGGCTGC
PheThrCys ProPheAsnGly AspCysArg IleThrLys AspAsnArgArg>

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      410      420      430      440      450
      *      *      *      *      *
CCACTGCCAG GCCTGCCGGC TCAAACGCTG TGTGGACATC GGCATGATGA
GGTGACGGTC CGGACGGCCG AGTTTGCGAC ACACCTGTAG CCGTACTACT
HisCysGln AlaCysArg LeuLysArgCys ValAspIle GlyMetMet>

      460      470      480      490      500
      *      *      *      *      *
AGGAGTTTCAT TCTGACAGAT GAGGAAGTGC AGAGGAAGCG GAGATGATC
TCCTCAAGTA AGACTGTCTA CTCCTTCACG TCTCCTTCGC CCTCTACTAG
LysGluPheIle LeuThrAsp GluGluVal GlnArgLysArg GluMetIle>

      510      520      530      540      550
      *      *      *      *      *
CTGAAGCGGA AGGAGGAGGA GGCCTTGAAG GACAGTCTGC GGCCTCAAGCT
GACTTCGCCT TCCTCCTCCT CCGGAAGTTC CTGTCAAGAC CCGGGTTCGA
LeuLysArg LysGluGluGlu AlaLeuLys AspSerLeu ArgProLysLeu>

      560      570      580      590      600
      *      *      *      *      *
GTCTGAGGAG CAGCAGCGCA TCATTGCCAT ACTGCTGGAC GCCCACCATA
CAGACTCCTC GTCGTCGCGT AGTAACGGTA TGACGACCTG CCGGTGGTAT
SerGluGlu GlnGlnArg IleIleAlaIle LeuLeuAsp AlaHisHis>

      610      620      630      640      650
      *      *      *      *      *
AGACCTACGA CCCCACCTAC TCCGACTTCT GCCAGTCCG GCCTCCAGTT
TCTGGATGCT GGGGTGGATG AGGCTGAAGA CCGTCAAGGC CCGAGGTCAA
LysThrTyrAsp ProThrTyr SerAspPhe CysGlnPheArg ProProVal>

      660      670      680      690      700
      *      *      *      *      *
CGTGTGAATG ATGGTGGAGG GAGCCATCCT TCCAGGCCCA ACTCCAGACA
GCACACTTAC TACCACCTCC CTCGGTAGGA AGGTCCGGGT TGAGGTCTGT
ArgValAsn AspGlyGlyGly SerHisPro SerArgPro AsnSerArgHis>

      710      720      730      740      750
      *      *      *      *      *
CACTCCCAGC TTCTCTGGGG ACTCCTCCTC CTCCTGCTCA GATCACTGTA
GTGAGGGTCG AAGAGACCCC TGAGGAGGAG GAGGACGAGT CTAGTGACAT
ThrProSer PheSerGly AspSerSerSer SerCysSer AspHisCys>

      760      770      780      790      800
      *      *      *      *      *
TCACCTCTTC AGACATGATG GACTCGTCCA GTTCTCCAA TCTGGATCTG
AGTGAGAGAAG TCTGTACTAC CTGAGCAGGT CGAAGAGGTT AGACCTAGAC
IleThrSerSer AspMetMet AspSerSer SerPheSerAsn LeuAspLeu>

      810      820      830      840      850
      *      *      *      *      *
AGTGAAGAAG ATTCAGATGA CCCTTCTGTG ACCCTAGAGC TGTCCAGCT
TCACTTCTTC TAAGTCTACT GCGAAGACAC TGGGATCTCG ACAGGGTCCA
SerGluGlu AspSerAspAsp ProSerVal ThrLeuGlu LeuSerGlnLeu>

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      860      870      880      890      900
      *      *      *      *      *
CTCCATGCTG CCCACCTGG CTGACCTGGT CAGTTACAGC ATCCAAAAGG
GAGGTACGAC GGGGTGGACC GACTGGACCA GTCAATGTCC TAGGTTTTCC
SerMetLeu ProHisLeu AlaAspLeuVal SerTyrSer IleGlnLys>

      910      920      930      940      950
      *      *      *      *      *
TCATTGGCTT TGCTAAGATG ATACCAGGAT TCAGAGACCT CACCTCTGAG
AGTAACCGAA ACGATTCTAC TATGGTCCTA AGTCTCTGGA GTGGAGACTC
ValIleGlyPhe AlaLysMet IleProGly PheArgAspLeu ThrSerGlu>

      960      970      980      990     1000
      *      *      *      *      *
GACCAGATCG TACTGCTGAA GTCAAGTGCC ATTGAGGTCA TCATGTTGCG
CTGGTCTAGC ATGACGACTT CAGTTCACGG TAACTCCAGT AGTACAACGC
AspGlnIle ValLeuLeuLys SerSerAla IleGluVal IleMetLeuArg>

     1010     1020     1030     1040     1050
      *      *      *      *      *
CTCCAATGAG TCCTTCACCA TGGACGACAT GTCCTGGACC TGTGGCAACC
GAGGTTACTC AGGAAGTGGT ACCTGCTGTA CAGSACCTGG ACACCGTTGG
SerAsnGlu SerPheThr MetAspAspMet SerTrpThr CysGlyAsn>

     1060     1070     1080     1090     1100
      *      *      *      *      *
AAGACTACAA GTACCGCGTC AGTGACGTGA CCAAAGCCGG ACAJAGCCTG
TTCTGATGTT CATGGCGCAG TCACTGCACT GGTTCGCGC TGTCTCGGAC
GlnAspTyrLys TyrArgVal SerAspVal ThrLysAlaGly HisSerLeu>

     1110     1120     1130     1140     1150
      *      *      *      *      *
GAGCTGATTG AGCCCTCAT CAAGTTCCAG GTGGGACTGA AGAAGCTGAA
CTCGACTAAC TCGGGGAGTA GTTCAAGGTC CACCTGACTT TCTTCGACTT
GluLeuIle GluProLeuIle LysPheGln ValGlyLeu LysLysLeuAsn>

     1160     1170     1180     1190     1200
      *      *      *      *      *
CTTGCAATGAG GAGGAGCATG TCCTGCTCAT GGCCATCTGC ATCGTCTCCC
GAACGTACTC CTCCTCGTAC AGGACGAGTA CCGGTAGACG TAGCAGAGGG
LeuHisGlu GluGluHis ValLeuLeuMet AlaIleCys IleValSer>

     1210     1220     1230     1240     1250
      *      *      *      *      *
CAGATCGTCC TGGGGTGACG GACGCCGCGC TGATTGAGGC CATCCAGGAC
GTCTAGCAGG ACCCCACGTC CTGCGGCGCG ACTAACTCCG GTAGGTCCTG
ProAspArgPro GlyValGln AspAlaAla LeuIleGluAla IleGlnAsp>

     1260     1270     1280     1290     1300
      *      *      *      *      *
CGCCTGTCCA ACACACTGCA GACGTACATC CGCTGCCGCC ACCCGCCCCC
GCGGACAGGT TGTGTGACGT CTGCATGTAG GCGACGGCGG TGGGCGGGGG
ArgLeuSer AsnThrLeuGln ThrTyrIle ArgCysArg HisProProPro>

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      1310      1320      1330      1340      1350
      *      *      *      *      *      *      *
GGGCAGCCAC CTGCTCTATG CCAAGATGAT CCAGAAGCTA GCCGACCTGC
CCCGTCGGTG GACGAGATAC GGTCTACTA GGTCTTCGAT CCGCTGGACG
GlySerHis LeuLeuTyr AlaLysMetIle GlnLysLeu AlaAspLeu>

      1360      1370      1380      1390      1400
      *      *      *      *      *      *      *
GCAGCCTCAA TGAGGAGCAC TCCAAGCAGT ACCGCTGCCT CTCCTTCCAG
CGTCGGAGTT ACTCCTCGTG AGGTTCGTCA TGGCGACGGA GAGGAAGGTC
ArgSerLeuAsn GluGluHis SerLysGln TyrArgCysLeu SerPheGln>

      1410      1420      1430      1440      1450
      *      *      *      *      *      *      *
CCTGAGTGCA GCATGAAGCT AACGCCCCTT GTGCTCGAAG TGTTTGGCAA
GGACTCACGT CGTACTTCGA TTGCGGGGAA CACGAGCTTC ACAAACCGTT
ProGluCys SerMetLysLeu ThrProLeu ValLeuGlu ValPheGlyAsn>

      1460
      *      *
TGAGATCTCC TGA
ACTCTAGAGG ACT
GluIleSer ***>
```

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FIGURE 6 TRANSCRIPT 9

(Sequence Range: 1 to 1382)

```

      10      20      30      40      50
      *      *      *      *      *
GTTTCCTTCT TCTGTCGGGG CGCCTTGGCA TGGAGTGGAG GAATAAGAAA
CAAAGGAAGA AGACAGCCCC GCGGAACCGT ACCTCACCTC CTTATTCTTT
                               MetGluTrpArg AsnLysLys>

      60      70      80      90     100
      *      *      *      *      *
AGGAGCGATT GGCTGTCGAT GGTGCTCAGA ACTGCTGGAG TGGAGGGGAT
TCCTCGCTAA CCGACAGCTA CCACGAGTCT TGACGACCTC ACCTCCCTTA
ArgSerAsp TrpLeuSerMet ValLeuArg ThrAlaGly ValGluGlyMet>

     110     120     130     140     150
      *      *      *      *      *
GGAGGCAATG GCGGCCAGCA CTTCCCTGCC TGACCCTGGA GACTTTGACC
CCTCCGTTAC CGCCGTCGT GAAGGGACGG ACTGGGACCT CTGAAACTGG
GluAlaMet AlaAlaSer ThrSerLeuPro AspProGly AspPheAsp>

     160     170     180     190     200
      *      *      *      *      *
GGAACGTGCC CCGGATCTGT GGGGTGTGTG GAGACCGAGC CACTGGCTTT
CCTTGACGG GGCCTAGACA CCCACACAC CTCTGGCTCG GTGACCGAAA
ArgAsnValPro ArgIleCys GlyValCys GlyAspArgAla ThrGlyPhe>

     210     220     230     240     250
      *      *      *      *      *
CACTTCAATG CTATGACCTG TGAAGGCTGC AAAGGCTTCT TCAGGCGAAG
GTGAAGTTAC GATACTGGAC ACTCCGACG TTTCCGAAGA AGTCCGCTTC
HisPheAsn AlaMetThrCys GluGlyCys LysGlyPhe PheArgArgSer>

     260     270     280     290     300
      *      *      *      *      *
CATGAAGCGG AAGGCACTAT TCACCTGCCC CTTCAACGGG GACTGCCGCA
GTACTTCGCC TTCCGTGATA AGTGGACGGG GAAAGTTGCC CTGACGGCGT
MetLysArg LysAlaLeu PheThrCysPro PheAsnGly AspCysArg>

     310     320     330     340     350
      *      *      *      *      *
TCACCAAGGA CAACCGACGC CACTGCCAGG CCTGCCGGCT CAAACGCTGT
AGTGGTTTCT GTTGGCTGCG GTGACGGTCC GGACGGCCGA GTTTCGACAA
IleThrLysAsp AsnArgArg HisCysGln AlaCysArgLeu LysArgCys>

     360     370     380     390     400
      *      *      *      *      *
GTGGACATCG GCATGATGAA GGAGTTCATT CTGACAGATG AGGAAGTGCA
CACCTGTAGC CGTACTACTT CCTCAAGTAA GACTGTCTAC TCCTTCACGT
ValAspIle GlyMetMetLys GluPheIle LeuThrAsp GluGluValGln>

```

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410 420 430 440 450
* * * * *
GAGGAAGCGG GAGATGATCC TGAAGCGGAA GGAGGAGGAG GCCTTGAAGG
CTCCTTCGCC CTCTACTAGG ACTTCGCCTT CCTCCTCCTC CGGAACCTCC
ArgLysArg GluMetIle LeuLysArgLys GluGluGlu AlaLeuLys>

460 470 480 490 500
* * * * *
ACAGTCTGCG GCCCAAGCTG TCTGAGGAGC AGCAGCGCAT CATTGCCATA
TGTCAGACGC CGGGTTCGAC AGACTCCTCG TCGTCGCGTA GTAACGGTAT
AspSerLeuArg ProLysLeu SerGluGlu GlnGlnArgIle IleAlaIle>

510 520 530 540 550
* * * * *
CTGTGGAACG CCCACCATAA GACCTACGAC CCCACCTACT CCGACTTCTG
GACGACCTGC GGGTGGTATT CTGGATGCTG GGGTGGATGA GGCTGAAGAC
LeuLeuAsp AlaHisHisLys ThrTyrAsp ProThrTyr SerAspPheCys>

560 570 580 590 600
* * * * *
CCAGTTCGGG CCTCCAGTTC GTGTGAATGA TGGTGGAGGG AGCCATCCTT
GGTCAAGGCC GGAGGTCAAG CACACTTACT ACCACCTCCC TCGGTAGGAA
GlnPheArg ProProVal ArgValAsnAsp GlyGlyGly SerHisPro>

610 620 630 640 650
* * * * *
CCAGGCCCAA CTCCAGACAC ACTCCCAGCT TCTCTGGGGA CTCCTCCTCC
GGTCCGGGTT GAGGTCTGTG TGAGGGTCTGA AGAGACCCCT GAGGAGGAGG
SerArgProAsn SerArgHis ThrProSer PheSerGlyAsp SerSerSer>

660 670 680 690 700
* * * * *
TCCTGCTCAG ATCACTGTAT CACCTCTTCA GACATGATGG ACTCGTCCAG
AGGACGAGTC TAGTGACATA GTGGAGAAGT CTGTACTACC TGAGCAGGTC
SerCysSer AspHisCysIle ThrSerSer AspMetMet AspSerSerSer>

710 720 730 740 750
* * * * *
CTTCTCCAAT CTGGATCTGA GTGAAGAAGA TTCAGATGAC CCTTCTGTGA
GAAGAGGTTA GACCTAGACT CACTTCTTCT AAGTCTACTG GGAAGACACT
PheSerAsn LeuAspLeu SerGluGluAsp SerAspAsp ProSerVal>

760 770 780 790 800
* * * * *
CCCTAGAGCT GTCCCAGCTC TCCATGCTGC CCCACCTGGC TGACCTGGTC
GGGATCTCGA CAGGGTCGAG AGGTACGACG GGGTGGACCG ACTGGACCAG
ThrLeuGluLeu SerGlnLeu SerMetLeu ProHisLeuAla AspLeuVal>

810 820 830 840 850
* * * * *
AGTTACAGCA TCCAAAAGGT CATTGGCTTT GCTAAGATGA TACCAGGATT
TCAATGTCTG AGGTTTTCCTA GTAACCGAAA CGATTCTACT ATGGTCCTAA
SerTyrSer IleGlnLysVal IleGlyPhe AlaLysMet IleProGlyPhe>

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      860      870      880      890      900
      *      *      *      *      *
CAGAGACCTC ACCTCTGAGG ACCAGATCGT ACTGCTGAAG TCAAGTGCCA
GTCTCTGGAG TGGAGACTCC TGGTCTAGCA TGACGACTTC AGTTCACGGT
ArgAspLeu ThrSerGlu AspGlnIleVal LeuLeuLys SerSerAla>

      910      920      930      940      950
      *      *      *      *      *
TTGAGGTCAT CATGTTGCCG TCCAATGAST CCTTCACCAT GGACGACATG
AACTCCAGTA GTACAACGCG AGGTTACTCA GGAAGTGATA CCTGCTGTAC
IleGluValIle MetLeuArg SerAsnGlu SerPheThrMet AspAspMet>

      960      970      980      990     1000
      *      *      *      *      *
TCCTGGACCT GTGGCAACCA AGACTACAAG TACCGCGTCA GTGACGTGAC
AGGACCTGGA CACCGTTGGT TCTGATGTTC ATGGCGCAGT CACTGCACGT
SerTrpThr CysGlyAsnGln AspTyrLys TyrArgVal SerAspValThr>

     1010     1020     1030     1040     1050
      *      *      *      *      *
CAAAGCCGGA CACAGCCTGG AGCTGATTGA GCCCCTCATC AAGTTCACGG
GTTTCGGCCT GTGTCGGACC TCGACTAATT CGGGGAGTAG TTCAAGGTCC
LysAlaGly HisSerLeu GluLeuIleGlu ProLeuIle LysPheGln>

     1060     1070     1080     1090     1100
      *      *      *      *      *
TGGGACTGAA GAAGCTGAAC TTGCATGAGG AGGAGCATGT CCTGCTCATG
ACCTTGACTT CTTCGACTTG AACGTACTTC TCCTCGTACA GGACGAGTAC
ValGlyLeuLys LysLeuAsn LeuHisGlu GluGluHisVal LeuLeuMet>

     1110     1120     1130     1140     1150
      *      *      *      *      *
GCCATCTGCA TCGTCTCCCC AGATCGTCTT GGGGTGAGG ACGGCGCGCT
CGGTAGACGT AGCAGAGGGG TCTAGCAGGA CCCACCTCC TGCGGCGCGA
AlaIleCys IleValSerPro AspArgPro GlyValGln AspAlaAlaLeu>

     1160     1170     1180     1190     1200
      *      *      *      *      *
GATTGAGGTC ATCCAGGACC GCCTGTCCAA CACACTGCAG ACGTACATCC
CTAACTCCGG TAGGTCTCTG CGGACAGGTT GTGTGACGTC TGCATGTAGG
IleGluAla IleGlnAsp ArgLeuSerAsn ThrLeuGln ThrTyrIle>

     1210     1220     1230     1240     1250
      *      *      *      *      *
GCTGCCGCCA CCCGCCCCCG GGCAGCCACC TGCTCTATGC CAAGATGATC
CGACGCGCGT GGGCGGGGGC CCGTCGGTGG ACGATATACG GTTCTACTAG
ArgCysArgHis ProProPro GlySerHis LeuLeuTyrAla LysMetIle>

     1260     1270     1280     1290     1300
      *      *      *      *      *
CAGAAGCTAG CCGACCTGCG CAGCCTCAAT GAGGAGCACT CCAAGCAGTA
GTCTTCGATC GGCTGGACGC GTCGGAGTTA CTCCTCGTGA GGTTCTGTCAT
GlnLysLeu AlaAspLeuArg SerLeuAsn GluGluHis SerLysGlnTyr>

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      1310      1320      1330      1340      1350
      *      *      *      *      *      *      *
CCGCTGCCTC TCCTTCCAGC CTGAGTGCAG CATGAAGCTA ACGCCCCTTG
GGCGACGGAG AGGAAGGTCG GACTCACGTC GTACTTCGAT TGCGGGGAAC
ArgCysLeu SerPheGln ProGluCysSer MetLysLeu ThrProLeu>

      1360      1370      1380
      *      *      *      *      *      *
TGCTCGAAGT GTTTGGCAAT GAGATCTCCT GA
ACGAGCTTCA CAAACCGTTA CTCTAGAGGA CT
ValLeuGluVal PheGlyAsn GluIleSer ***>
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FIGURE 7 TRANSCRIPT 10

(Sequence Range: 1 to 1534)

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      10      20      30      40      50
      *      *      *      *      *
GTTTCCTTCT TCTGTCGGGG CGCCTTGGCA TGGAGTGGAG GAATAAGAAA
CAAAGGAAGA AGACAGCCCC GCGGAACCGT ACCTCACCTC CTTATTCTTT
                               MetGluTrpArg AsnLysLys>

      60      70      80      90     100
      *      *      *      *      *
AGGAGCGATT GGCTGTCGAT GGTGCTCAGA ACTGCTGGAG TGGAGGGGAT
TCCTCGCTAA CCGACAGCTA CCACGAGTCT TGACGACCTC ACCTCCCCTA
ArgSerAsp TrpLeuSerMet ValLeuArg ThrAlaGly ValGluGlyMet>

     110     120     130     140     150
      *      *      *      *      *
GGAGGCAATG GCGGCCAGCA CTTCCCTGCC TGACCCTGGA GACTTTGACC
CCTCCGTTAC CGCCGGTTCGT GAAGGGACGG ACTGGGACCT CTGAAACTGG
GluAlaMet AlaAlaSer ThrSerLeuPro AspProGly AspPheAsp>

     160     170     180     190     200
      *      *      *      *      *
GGAACGTGCC CCGGATCTGT GGGGIGTGTG GAGACCGAGC CACTGGCTTT
CCTTGACCGG GGCCTAGACA CCCACACAC CTCTGGCTCG GTGACCGAAA
ArgAsnValPro ArgIleCys GlyValCys GlyAspArgAla ThrGlyPhe>

     210     220     230     240     250
      *      *      *      *      *
CACTTCAATG CTATGACCTG TGAAGGCTGC AAAGGCTTCT TCAGGTGAGC
GTGAAGTTAC GATACTGGAC ACTTCCGACG TTTCCGAAGA AGTCCACTCG
HisPheAsn AlaMetThrCys GluGlyCys LysGlyPhe PheArg***

     260     270     280     290     300
      *      *      *      *      *
CCCCCTCCCA GGCTCTCCCC AGTGGAAAGG GAGGGAGAAG AAGCAAGGTG
GGGGGAGGGT CCGAGAGGGG TCACCTTTCC CTCCCTCTTC TTCGTTCCAC

     310     320     330     340     350
      *      *      *      *      *
TTTCCATGAA GGGAGCCCTT GCATTTTTCa CATCTCCTTC CTTACAATGT
AAAGGTACTT CCCTCGGGAA CGTAAAAAGT GTAGAGGAAG GAATGTTACA

     360     370     380     390     400
      *      *      *      *      *
CCATGGAACA TGCGGCGCTC ACAGCCACAG GAGCAGGAGG GTCTTGGCGA
GGTACCTTGT ACGCCGCGAG TGTCGGTGTC CTCGTCCTCC CAGAACCGCT

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      410      420      430      440      450
      *      *      *      *      *
ACCATGAAGC GGAAGGCACT ATTCACCTGC CCCTTCAACG GGGACTGCCG
TCGTACTTCG CCTTCCGTGA TAAGTGGACG GGAAGTTGT CCCTGACGGC

      460      470      480      490      500
      *      *      *      *      *
CATCACCAAG GACAACCGAC GCCACTGCCA GGCCTGCCCG CTCAAACGCT
GTAGTGGTTC CTGTTGGCTG CCGTGACGGT CCGGACGGCC GAGTTTGGA

      510      520      530      540      550
      *      *      *      *      *
GTGTGGACAT CGGCATGATG AAGGAGTTCA TTCTGACAGA TGAGGAAGTG
CACACCTGTA GCCGTACTAC TTCCTCAAGT AAGACTGTCT ACTCCTTCAC

      560      570      580      590      600
      *      *      *      *      *
CAGAGGAAGC GGGAGATGAT CCTGAAGCGG AAGGAGGAGG AGGCCTTGAA
GTCTCCTTCG CCCTCTACTA GGACTTCGCC TTCCTCCTCC TCCGGAACCT

      610      620      630      640      650
      *      *      *      *      *
GGACAGTCTG CGGCCCAAGC TGTCTGAGGA GCAGCAGCGE ATCATTGCCA
CCTGTCAGAC GCCGGGTTCG ACAGACTCCT CGTCGTCCGG TAGTAACGGT

      660      670      680      690      700
      *      *      *      *      *
TACTGCTGGA CGCCCACCAT AAGACCTACG ACCCCACCTA CTCGCACTTC
ATGACGACCT GCGGGTGGTA TTCTGGATGC TGGGGTGGAT GAGSCTGAAG

      710      720      730      740      750
      *      *      *      *      *
TGCCAGTTCC GGCCTCCAGT TCGTGTGAAT GATGGTGGAG GGAGCCATCC
ACGGTCAAGG CCGGAGGTCA AGCACACTTA CTACCACCTC CCTCGGTAGG

      760      770      780      790      800
      *      *      *      *      *
TTCCAGGCCC AACTCCAGAC AACTCCACAG CTTCTCTGGG GACTCCTCCT
AAGGTCCGGG TTGAGGTCTG TGTGAGGGTC GAAGAGACCC CTGAGGAGGA

      810      820      830      840      850
      *      *      *      *      *
CCTCCTGCTC AGATCACTGT ATCACCTCTT CAGACATGAT GGACTCGTCC
GGAGGACGAG TCTAGTGACA TAGTGGAGAA GTCTGTACTA CCTGAGCAGG

      860      870      880      890      900
      *      *      *      *      *
AGCTTCTCCA ATCTGGATCT GAGTGAAGAA GATTGAGATG ACCCTTCTGT
TCGAAGAGGT TAGACCTAGA CTCACTTCTT CTAAGTCTAC TGGGAAGACA

      910      920      930      940      950
      *      *      *      *      *
GACCCTAGAG CTGTCCCAGC TCTCCATGCT GCCCCACCTG GCTGACCTGG
CTGGGATCTC GACAGGGTCG AGAGGTACGA CGGGGTGGAC CGACTGGACC

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      960      970      980      990      1000
      *      *      *      *      *
TCAGTTACAG CATCCAAAAG GTCATTGGCT TTGCTAAGAT GATACCAGGA
AGTCAATGTC GTAGGTTTTC CAGTAACCGA AACGATTCTA CTATGGTCCT

      1010      1020      1030      1040      1050
      *      *      *      *      *
TTCAGAGACC TCACCTCTGA GGACCAGATC GTACTGCTGA AGTCAAGTGC
AAGTCTCTGG AGTGGAGACT CCTGGTCTAG CATGACGACT TCAGTTCACG

      1060      1070      1080      1090      1100
      *      *      *      *      *
CATTGAGGTC ATCATGTTGC GCTCCAATGA GTCCTTCACC ATGGACGACA
GTAAGTCCAG TAGTACAACG CGAGGTTACT CAGGAAGTGG TACCTGCTGT

      1110      1120      1130      1140      1150
      *      *      *      *      *
TGTCCTGGAC CTGTGGCAAC CAAGACTACA AGTACCGCGT CAGTGACGTG
ACAGGACCTG GACACCGTTG GTTCTGATGT TCATGGCGCA GTCAGTGCAC

      1160      1170      1180      1190      1200
      *      *      *      *      *
ACCAAAGCCG GACACAGCCT GGAGCTGATT GAGCCCCCTCA TCAAGTCCCA
TGTTTTCGGC CTGTGTCGGA CCTCGACTAA CTCGGGAGGT AGTTCAAGGT

      1210      1220      1230      1240      1250
      *      *      *      *      *
GGTGGGACTG AAGAAGCTGA ACTTGCATGA GGAGGAGCAT GTCCTGCTCA
CCACCCTGAC TTCTTCGACT TGAACGTACT CTCCTTCGTA CAGGACGAGT

      1260      1270      1280      1290      1300
      *      *      *      *      *
TGGCCATCTG CATCGTCTCC CCAGATCGTC CTGGGCTGCA GGACGCCGCG
ACCGGTAGAC GTAGCAGAGG GGTCTAGCAG GACCCACGT CCTGCGGCGC

      1310      1320      1330      1340      1350
      *      *      *      *      *
CTGATTGAGG CCATCCAGGA CCGCCTGTCC AACACACTGC AGACGTACAT
GACTAACTCC GGTAGGTCCT GGCGGACAGG TTGTGTGACG TCTGCATGTA

      1360      1370      1380      1390      1400
      *      *      *      *      *
CCGCTGCCGC CACCCGCCCC CGGGCAGCCA CCTGCTCTAT GCCAAGATGA
GGCGACGGCG GTGGGCGGGG GCCCGTCGGT GGACGAGATA CGGTTCTACT

      1410      1420      1430      1440      1450
      *      *      *      *      *
TCCAGAAGCT AGCCGACCTG CGCAGCCTCA ATGAGGAGCA CTCCAAGCAG
AGGTCTTCGA TCGGCTGGAC GCGTCGSAGT TACTCCTCGT GAGGTTCTGC

      1460      1470      1480      1490      1500
      *      *      *      *      *
TACCGCTGCC TCTCCTTCCA GCCTGASTGC AGCATGAAGC TAACGCCCCCT
ATGGCGACGG AGAGGAAGGT CGGACTCAGC TCGTACTTCG ATTGCGGGGA

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1510	1520	1530
TGTGCTCGAA	GTGTTTGGCA	ATGAGATCTC
CTGA		
ACACGAGCTT	CACAAACCGT	TACTCTAGAG
GA		

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FIGURE 8 TRANSCRIPT 11

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      10      20      30      40      50
      *      *      *      *      *
TSCGACCTTG GCGGTGAGCC TGGGGACAGG GGTGAGGCCA GAGACGGACG
ACGCTGGAAC CGCCACTGGG ACCCCTGTCC CCACTCGGGT CTCTGCTGSD

      60      70      80      90     100
      *      *      *      *      *
GACGCAGGGG CCGGGCCCAA GCGGAGGSAG AACAGCGGCA CTAAGGCAGA
CTCGTCCCG CCGCCGGGTT CCGCTCCGTC TTGTGGGGT GATTCCGTCT

     110     120     130     140     150
      *      *      *      *      *
AAGGAAGAGG GCGGTGTGTT CACCCCGACC CCAATCCATC ACTCAGCAAC
TTCTTCTCC CCGCACACAA GTGGGGGTGG GGTIAGGTAG TGAGTCGTTG

     160     170     180     190     200
      *      *      *      *      *
TCCTAGACGE TGSTAGAAAG TTCTTCCBAG GAGCCTGCCA TCCAGTCGTG
AGGATCTGCG ACCATCTTTC AAGGAGGCTC CTCGACGGT AGSTCAGCAC

     210     220     230     240     250
      *      *      *      *      *
CGTGCGAAG CCTTGGGTC TGAAGTGTCT GTGACGCTC ACAGAAGAGC
GCACGTCTTC GGAAACCCAG ACTTCACAGA CACTCTGGAG TGCTTCTCG

     260     270     280     290     300
      *      *      *      *      *
ACCCCTGGGC TCCACTTACC TSCCCCTGC TCCTTCAGGG ATGGAGGCAG
TGGGGACCCG AGGTGAATGG ACGGGGGACG AGGAAGTCCC TACCTCCGTT
                                         MetGluAla>

     310     320     330     340     350
      *      *      *      *      *
TGGCGGCCAG CATTCCCTG CCGTGAJCTG GAGACITGA CCGGAACGTG
ACCGCCGGTC GTGAAGGGAC GGACTGGGAT CTCTGAACT GGCCTTGCAC
MetAlaAlaSer ThrSerLeu ProAspPro GlyAspPheAsp ArgAsnVal>

     360     370     380     390     400
      *      *      *      *      *
CCCCGGATCT GTGGGGTGTG TGCAGACCGA GCCACTGGCT TTCACTTCAA
GGGGCCTAGA CACCCACAC ACCTCTGGCT CGGTGACCGA AAGTGAAGTT
ProArgIle CysGlyValCys GlyAspArg AlaThrGly PheHisPheAsn>

     410     420     430     440     450
      *      *      *      *      *
TGCTATGACC TGTGAAGGCT GCAAAGGCTT CTTCAGGCGA AGCATGAAGC
ACGATACTGG ACACTTCGA CGTTTCCGAA GAAGTCCGCT TCGTACTTGG
AlaMetThr CysGluGly CysLysGlyPhe PheArgArg SerMetLys>

     460     470     480     490     500
      *      *      *      *      *
GGAAGGCACT ATTCACCTGC CCCTTCAACG GGGACTGCCG CATCACCAAG
CCTTCCGTGA TAAGTGGACG GGGAAATTGC CCCTGACGGC GTAGTGGTTC
ArgLysAlaLeu PheThrCys ProPheAsn GlyAspCysArg IleThrLys>

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      510      520      530      540      550
      *      *      *      *      *
GACAACCGAC GCCACTGCCA GGCTGCGCG CTCAAACGCT GTGTGGACAT
CTGTTGGGTG CGGTGACGCT CCGGACGGCC GAGTTTGGCA CACACCTGTA
AspAsnArg ArgHisCysGln AlaCysArg LeuLysArg CysValAspIle>

      560      570      580      590      600
      *      *      *      *      *
CGGCATGATG AAGCASTTCA TTCTGACAGA TGAGGAAGTG CAGAGGAAGC
GCCGTAATAA TTCTCAAGT AAGACTGTCT ACTCCTTCAC GTCTCCTTCG
GlyMetMet LysGluPhe IleLeuThrAsp GluGluVal GlnArgLys>

      610      620      630      640      650
      *      *      *      *      *
GGGAGATGAT CCTGAAGCGG AAGGAGGAGG AGGCCTTGAA GGACAGTCTG
CGCTCTAATA GGACTTCGGC TTCTCCTCC TCGGAACTT CCGTCAGAG
ArgGluMetIle LeuLysArg LysGluGlu GluAlaLeuLys AspSerLeu>

      660      670      680      690      700
      *      *      *      *      *
CGGCCCAAGC TGTCTGAGGA GCAGCAGCGC ATCATTGCCA TATTGCTGGA
GUCGGGTTCG ACAGACTCCT CGTCGTCGCG TAGTAACGGT ATGACGACCT
ArgProLys LeuSerGluGlu GlnGlnArg IleIleAla IleLeuLeuAsp>

      710      720      730      740      750
      *      *      *      *      *
CGCCCAACAT AAGACCTAGG ACCCCACCTA CTCGACTTC TGCCAGTTCC
GCGGGTGSTA TTCTGGATGC TGGGGTGGAT GAGGCTGAAG ACGGTCAAGG
AlaHisHis LysThrTyr AspProThrTyr SerAspPhe CysGlnPhe>

      760      770      780      790      800
      *      *      *      *      *
GGCCTCCAGT TCGTGTGAAT GATGGTGGAG GGAGCCATCC TTCCAGGCCG
CUGGAGGTCA AGCACACTTA CTACCACCTC CTTGGTAGG AAGGTCCGGG
ArgProProVal ArgValAsn AspGlyGly GlySerHisPro SerArgPro>

      810      820      830      840      850
      *      *      *      *      *
AACTCCAAAT ACACTCCAG CTTCTCTGGG GACTGCTCCT GTCTCTGCTC
TTGAGGTCTG TGTGAGGGTC GAAGAGACCC CTGAGGAGGA GGAGGACGAG
AsnSerArg HisThrProSer PheSerGly AspSerSer SerSerCysSer>

      860      870      880      890      900
      *      *      *      *      *
AGATCACTGT ATCAGCTCTT CAGACATGAT GGAATCGTCC AGCTTCTCCA
TCTAGTGACA TAGTGAGAA GTCTGTACTA CTTGAGCAGG TCGAAGAGGT
AspHisCys IleThrSer SerAspMetMet AspSerSer SerPheSer>

      910      920      930      940      950
      *      *      *      *      *
ATCTGGATCT GAGTGAAGAA GATTCAGATG ACCCTTCTGT GACCCTAGAG
TAGACCTAGA CTCACTTCTT CTAAGTCTAC TGGGAAGACA CTGGGATCTC
AsnLeuAspLeu SerGluGlu AspSerAsp AspProSerVal ThrLeuGlu>

      960      970      980      990      1000
      *      *      *      *      *
CTGTCCAGAG TCTCCATGCT GCCCACCTG GTTACCTGG TCAGTTACAG
GACAGGCTCG AGAGGTACGA CGGGGTGGAC GCACTGGACC AGTCAATGT
LeuSerGln LeuSerMetLeu ProHisLeu AlaAspLeu ValSerTyrSer>

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1010      1020      1030      1040      1050
*         *         *         *         *
CATCCAAAAG GTCATTGGCT TTGCTAAGAT GATACCAAGG TTCAGAGACC
GTAGGTTTTT CAGTAACCGA AACGATTCTA CTATGGTCTT AAGTCTCTGG
IleGlnLys ValIleGly PheAlaLysMet IleProGly PheArgAsp>

1060      1070      1080      1090      1100
*         *         *         *         *
TCACCTCTGA GGACCAGATC GTACTGCTGA AGTCAAGTGC CATTGAGGTC
AGTGGAGACT CCTGGTCTAG CATGACGACT TCAGTTCAAG GTAATTCCAG
LeuThrSerGlu AspGlnIle ValLeuLeu LysSerSerAla IleGluVal>

1110      1120      1130      1140      1150
*         *         *         *         *
ATCATGTTGC GGTCCAATGA GTCCTTCACC ATGGACGACA TGTCTGGGAC
TAGTACAACG CGAGGTTACT CAGGAAGTGG TACCTGCTGT ACAGGACCTG
IleMetLeu ArgSerAsnGlu SerPheThr MetAspAsp MetSerTrpThr>

1160      1170      1180      1190      1200
*         *         *         *         *
CTGTGGCAAC CAAGACTATA AGTAGGCGCT CAGTGACGTG ACCAAAGCCG
GACACCGTTG GTTCTGATGT TCATGGCGCA GTCAGTGCAC TGGTTTCGGC
CysGlyAsn GlnAspTyr LysTyrArgVal SerAspVal ThrLysAla>

1210      1220      1230      1240      1250
*         *         *         *         *
GACACAGCCT GGAGCTGATT GAGCCCTCTA TCAAGTTTCA GGTGGGACTG
CTGTGTCGGA CCTCGACTAA CTCGGGGAGT AGTTCAAGGT CCACCCTGAC
GlyHisSerLeu GluLeuIle GluProLeu IleLysPheGln ValGlyLeu>

1260      1270      1280      1290      1300
*         *         *         *         *
AAGAAGCTGA ACTTGCAATG GGAGGAGCAT GTCTGCTCTA TGGCCATCTG
TTCTTCGACT TGAACGTAAT CCTCCTCTTA CAGGAGGAGT ACCGGTAGAA
LysLysLeu AsnLeuHisGlu GluGluHis ValLeuLeu MetAlaIleCys>

1310      1320      1330      1340      1350
*         *         *         *         *
CATCGTCTCC CCAGATCGTC CTGGGGTSCA GGACGGCGCG CTGATTGAGG
GTAGCAGAGG GGTCTAGCAG GACCCCAAGT CCTGCGGGCG GACTAACTCT
IleValSer ProAspArg ProGlyValGln AspAlaAla LeuIleGlu>

1360      1370      1380      1390      1400
*         *         *         *         *
CCATCCAGGA CCGCCTGTCC AACACACTGC AGACGTACAT CCGCTGCCCG
GGTAGGTCCT GCGGGACAGG TTGTGTGACG TCTGCATGTA GCGGACGGCG
AlaIleGlnAsp ArgLeuSer AsnThrLeu GlnThrTyrIle ArgCysArg>

1410      1420      1430      1440      1450
*         *         *         *         *
CACCCGCCCC CGGGCAGCCA CCTGCTCTAT GCCAAGATGA TCCAGAAGCT
GTGGGCGGGG GCGCGTCGGT GGACGAGATA CGGTCTTACT AAGTCTTCCA
HisProPro ProGlySerHis LeuLeuTyr AlaLysMet IleGlnLysLeu>

1460      1470      1480      1490      1500
*         *         *         *         *
AGCCGACCTG CGCAGCCTCA ATGAGGAGCA CTCCAAGCAG TACCGCTGCC
TCGGCTGGAC GCGTCGGAGT TACTCCTCGT GAGGTTGCTC ATGGCGACGG
AlaAspLeu ArgSerLeu AsnGluGluHis SerLysGln TyrArgCys>

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      1510      1520      1530      1540      1550
      *          *          *          *          *
TCTCCTTCCA GCCTGAGTGC AGCATGAAGC TAACGCCCTT TGTGCTCGAA
AGAGGAAGGT CGGACTCAGC TCGTACTTCG ATTGCGGGGA ACACGAGCTT
LeuSerPheGln ProGluCys SerMetLys LeuThrProLeu ValLeuGlu>
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      1560      1570
      *          *
GTGTTTGGCA ATGAGATCTC CTGA
CACAAACCGT TACTCTAGAG GACT
ValPheGly AsnGluIleSer ***>
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 98/00817

A. CLASSIFICATION OF SUBJECT MATTER		
Int Cl ⁶ : C12N 15/12; C07K 14/72; C07K 16/28; A01K 67/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) I/C as above		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Derwent WPAT, Medline : Vitamin D/Calcitriol receptor, sequence, Isoform/polymorphism/exon/variant. Sequence IDS 1-12: Swiss Prot, EMBL, PIR Genbank		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Baker RA et al. Proc Nat Acad Sci USA. 1988. 85 3294-3298 Whole document	1-15, 21-24
A	Goto H et al. Biochim Biophys Acta. 1992. 1132: 103-108 Whole document	1-15, 21-24
X	Miyamoto K-I et al Mol Endocrin. 1997. 11(8): 1165-1179 Whole document	1-24
P, X	Crofts LA et al. Proc Nat Acad Sci USA. 1998. 95: 10529-10534 Whole document	1-24
<input type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 29 October 1998		Date of mailing of the international search report - 9 NOV 1998
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (02) 6285 3929		Authorized officer GILLIAN ALLEN Telephone No.: (02) 6283 2266